Molecular Pharmacology

Supplemental Data

Evaluation of the Selectivity and Cysteine-Dependence of Inhibitors Across the Regulator of G Protein Signaling Family

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Figure S1. SDS-PAGE analysis of purified RH domains. A. Wild-type RH domains purified using IMAC. Number above well corresponds to RGS family member. B. SDS-PAGE analysis of mutant RH domains, where all Cys residues have been replaced with Ala. Number above well corresponds to RGS family member.
Figure S2
Figure S2. Inhibition of WT and Cys-null RGS RH domain interactions with Gαo. Concentration-response assessment of previously described inhibitors’ ability to disrupt RGS: Gαo PPI using AlphaScreen assay. Data for WT and Cys-null RH domains are summarized in Table 2 and Table 3, respectively. Data represent mean ± SD from n=3 independent experiments.
Figure S3. Inhibition of RGS RH domain: Goi1 interaction in cells. NanoBiT luminescence complementation assay in HEK293T cells expressing indicated RGS RH domain and Ga11, with the notable exception that RGS2 was co-expressed with Gaq. Decrease in signal in response to compound treatment as a percent of vehicle treatment. 31.6 μM CCG-4986 (A) and CCG-50014 at 7.5 μM (B) and 31.6 μM (C) do not result RGS: Ga inhibition that is discernible from assay inhibition (Control PPI). Data represent mean of n=3 independent experiments ± SD.
Table S1. RGS Inhibitors Without Detectable Inhibition of WT-RGS: Gαo Interaction. Biochemical characterization of RGS inhibitors for their ability to disrupt the WT-RGS: Gαo PPI using AlphaScreen assay. Data represent the IC\textsubscript{50} with the 95% CI in parentheses from n=3 independent experiments. Concentration response curves are shown in Supplementary Figure S2. NC indicates that an IC\textsubscript{50} value was not calculable.